WHO Prequalification of Vector Control Products

Long-term community studies for ITNs

Factors which may affect the validity of long-term community studies

- Community where the study takes place not suitable for where the product is intended to be deployed
- Limitations for periodic follow up in selected community, for example, unstable or migratory community resulting in high loss of distributed ITNs
- Inappropriate study design
- Chemical, physical and entomological characterisation testing not conducted in compliance with relevant standards (for example, GLP and/or ISO17025)
- Conduct of chemical, physical and entomological characterisation testing not in alignment with pre-market data generation
- Inadequate sample sizes due to:
    - Insufficient number of ITNs distributed,
    - Insufficient number of households visited
    - Insufficient number of nets inspected
    - High attrition of ITNs during study such that sufficient numbers of ITNs are not present in the study after two years
    - ITN distribution campaigns taking place during the study period, leading to replacement of study ITNs in households
    - Low mosquito densities (for semi-field analyses only)
- Local vector population not suitable for the intended use of the ITN product under investigation (for semi-field analyses only)

Requirements for long-term community studies

In order to demonstrate acceptability, durability and efficacy under three years of routine operational use, ITNs are expected to:

- Be sufficiently acceptable to the study community, and durable under routine conditions of use to demonstrate three years of functional ITN survival (median functional net survival should not be less than two years at the conclusion of the study);
- Demonstrate the physical and chemical consistency of the ITN fabric by means of chemical and physical analysis of sampled ITNs at baseline, 12, 18, 24 and 36 months;
- Demonstrate comparable entomological efficacy throughout the study by means of a non-inferiority analysis of semi-field study data using operationally aged nets of ages 0, 12, 24 and 36 months.

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2 Introduction

As established in the WHO Guideline for prequalification assessment of ITNs, the decision to prequalify an ITN is based on the substantiation of a reasonable expectation of product performance as assessed using a weight of evidence approach. Performance is defined in the guideline as the ability of the ITN to:

- provide continuous and controlled release of the active ingredient(s) (AIs) to maintain the intended effects of the product on target vectors over the intended useful life of the ITN, when used as instructed;
- maintain physical integrity for the duration of the intended useful life when used as instructed and protected from damage, for example, direct sunlight, open flame, animals/rodents, sharp objects, and excessive stretching.

The pre-market assessments play a pivotal role in predicting real-world performance. The pre-market assessments of the duration of product performance are based on data from artificially aged samples and predictive physical tests of fabric durability. This approach necessitates a careful balance between ensuring timely access to life-saving products and the need for robust, real-world data to characterize durations of performance in different settings.

Despite the value of pre-market data, real-world data collection remains essential for ITNs based on the expectations for a prolonged duration of efficacy under highly variable use settings and environmental conditions. Such data are instrumental in refining expectations for product performance across diverse use settings. Long-term community studies are fundamentally focused on the relationship between the product quality (formulation, manufacturing process, and physical/chemical characteristics including regeneration/wash resistance) and the performance of ITNs in operational settings. As a post-prequalification commitment for prequalified ITNs, these studies are requirements under Module 5 due to the inclusion of semi-field efficacy studies to connect the quality and efficacy of operationally aged ITNs.

This implementation guidance document is intended to provide information to manufacturers on the study design, generation of data, and reporting for long-term community studies. In developing product specific studies, deviations from this guidance may be necessary and justifiable. Manufacturers are encouraged to submit a PQ200 protocol review application prior to initiating studies. Deviations from this guidance should be clearly identified and a rationale provided.

3 Purpose of the Study

For the purposes of prequalification, long-term community studies are studies that are conducted using ITNs that have been distributed in communities and are thereby operationally aged through routine use.

The studies are conducted to investigate the acceptability to at-risk communities (evident through continued use), fabric integrity (rate at which ITNs accumulate damage), chemical/biological consistency (by means of the presentation of AI(s) in/on the fabric), physical consistency, and entomological efficacy under conditions of routine use throughout the intended usage period of the product, by means of:

Community assessment

- Assessment of continued net presence and use;
- Fabric integrity assessments to quantify damage to ITNs;
- User surveys to collect data on the environment in which the net is used as well as users' attitude to ITNs that may affect ITN survival;
- User surveys to collect data on adverse events.

Laboratory/semi-field assessment

- Chemical analysis to determine the retention of AI content;
- Bioassays to investigate the consistency of biological activity of the fabric's surface;
- Assessment of relevant ITN physical properties;
- Investigation of the biological activity of operationally aged ITNs under simulated user conditions by observing the relevant effects on free-flying mosquitoes.

Long-term community studies are conducted for a duration of 36 months or until fewer than 50% of ITNs in the test arm survive. Should fewer than 50% of ITNs in the test arm survive at an intermediate timepoint, the study should be stopped, and the 36-month testing requirements should be conducted using the intermediate timepoint as the final timepoint.

4 Requirement for submission of long-term community studies

It is a requirement that a minimum of two long-term community studies are submitted to PQT/VCP. These studies are part of the requirements for Module 5 of the product dossier, as described in the WHO Guideline for prequalification assessment of ITNs. Though studies may be included in the application for prequalification, it is generally expected that these studies will be submitted post-market as per post-PQ commitment requirements included in the prequalification decision.

Long-term community studies should be GLP compliant, where applicable, for example, for laboratory sub-studies. Attention must be paid to data integrity, follow up and investigator bias throughout.

5 Data requirements for long-term community studies

In order to demonstrate the continuity of ITN performance throughout the intended useful life of the product, long-term community studies are required to collect and present data on:

Community assessment:

- The presence or absence of ITNs in houses, i.e., hanging in use or present in the house unused vs. discarded/repurposed/disposed (attrition), as well as given away/used elsewhere/lost/stolen (lost to follow up) *(1-3);*
- The physical condition of the net (appearance, proportionate hole index);
- Covariates used to calculate ITN survival:
  - Environmental (sleeping space, house structure, socio-economic status (SES));
  - Behavioural (net attitude score, washing frequency, hanging, care and repair).

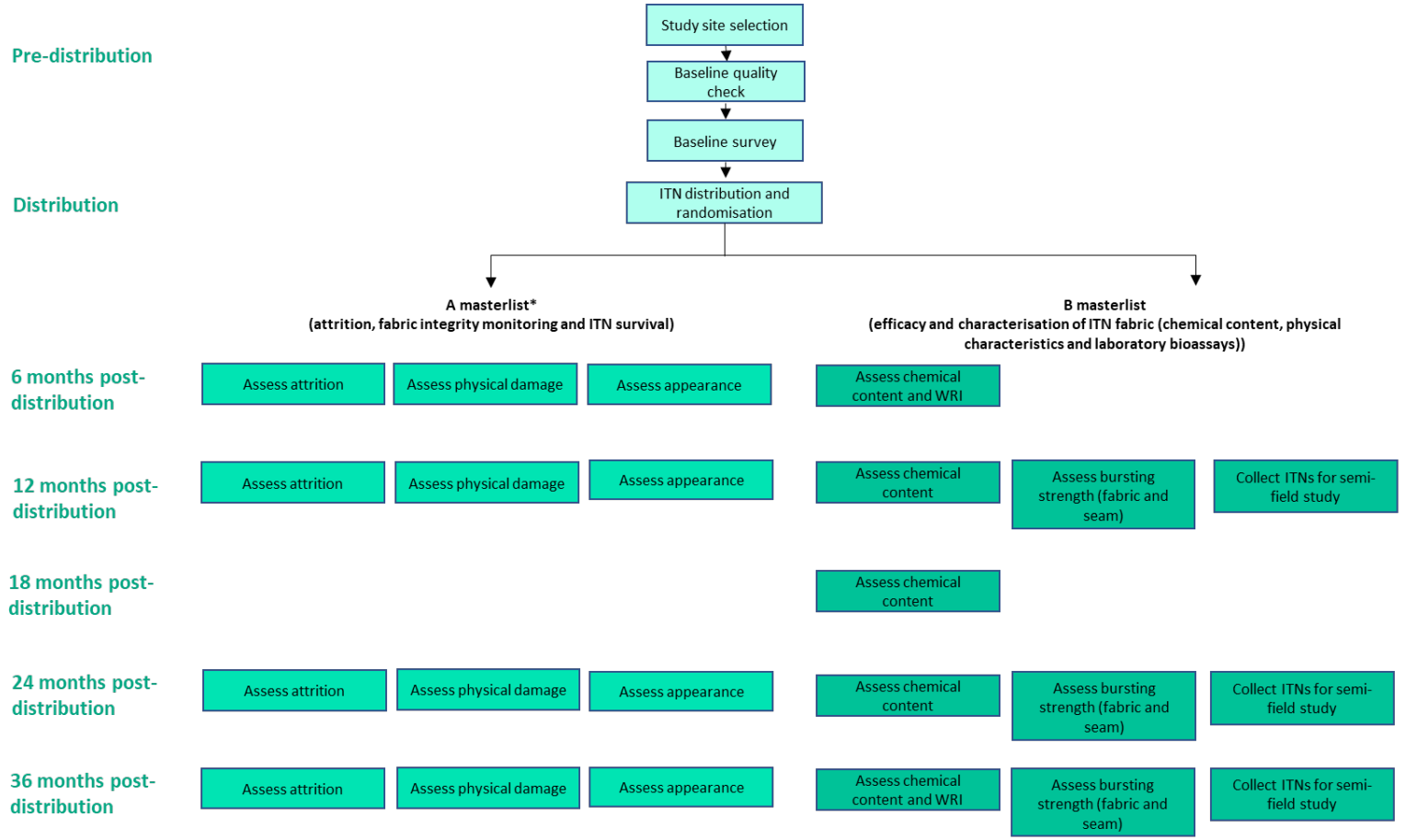
Laboratory/semi-field assessment:

- Consistency of the ITN fabric:
  - Chemical content measured by means of AI content;
  - Wash resistance index;
  - Fabric and seam bursting strength;
  - Consistency of insecticidal effect of the ITN fabric by means of bioassays on sampled; fabric (conducted at the time of the semi-field study);
- Entomological efficacy against free-flying mosquitoes.

5.1 Schematics for long-term community studies

Long-term community studies contain a number of sub-studies and should be designed according to the schematics presented in Figs 1 and 2 below. The sub-studies are divided into those that **do not** require destructive sampling and those that **do** require destructive sampling (list A and list B surveys, respectively). Refer to section 9.4 for further details on the A and B masterlists.

5.1.1 Overall study schematic

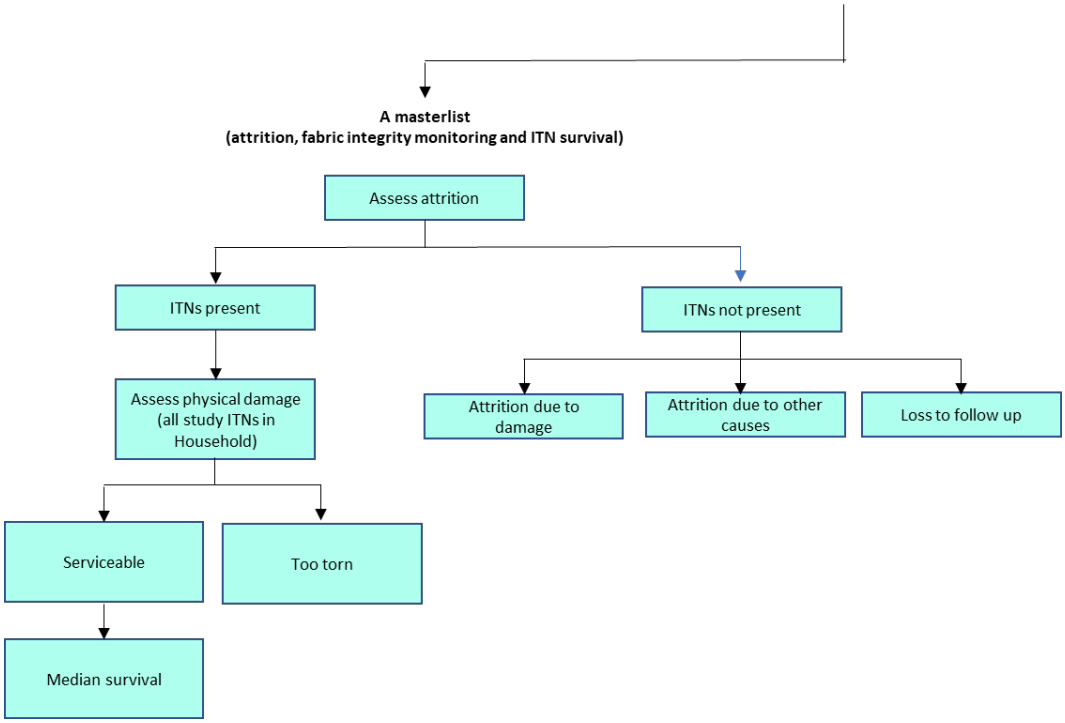


* The schematic presents attrition and physical damage separately for the purposes of demonstrating study timepoints. For further details of the attrition and physical damage monitoring cascade, refer to Fig 1B.

** Adapted from (3)

Figure 1. Schematic for long-term community studies

5.1.2 Attrition monitoring cascade



* Adapted from (3)

Figure 2. Attrition and fabric integrity cascade to be conducted at each follow up timepoint

6 Ethics considerations

Institutional ethical approval for the study must be sought from the responsible local/regional Ethical Review Board. Written informed consent must be obtained, in accordance with the prevailing rules and regulations, from each participating household prior to their participation in the study (refer to section 6.1 for further details).

6.1 Community sensitisation and informed consent

Applicants should ensure that all relevant permissions are obtained from relevant regional/district authorities prior to the final selection of a study site. The assistance of community leaders should be sought to obtain permission to use the community as a study site and to inform community members of the study's objectives and methods. Meetings with community representatives can encourage the adherence of study participants.

Informed written consent in the local language is required from all individuals and households who are participating in the study, at the time of the baseline census. For members of the household who cannot read, the informed consent form should be read out and explained to them by a member of the study team in the local language in the presence of literate witnesses from the community. Once they have

consented, these people will be asked to mark a thumb or finger impression on the form and the witness will be asked to sign it. One copy of the form should be left with the household for their personal records.

The risks and benefits of participation in the study must be explained to potential participants and they must be informed that they can refuse to participate in the follow-up interview(s) (or refuse to answer specific questions during the follow up interviews) and still keep their ITN. All study participants should be advised to contact the study focal person should they experience an adverse event.

In accordance with best practices, all study participants should be advised to seek medical care at the nearest health facility if they observe any sign or symptom of malaria or other vector-borne disease and any adverse effects of using ITNs.

6.2 Semi-field study

For the semi-field sub-study, as is standard practice, institutional ethical approval for the study must be sought from the local/regional Ethical Review Board (this may be obtained as part of the ethics application for the whole community study). Written informed consent must be obtained from each volunteer sleeper prior to their participation in the study after the risks and benefits of participation in the study have been explained. It must be explained to volunteers that they may withdraw their participation in the study at any point without any negative consequences.

Volunteer sleepers should be monitored for possible adverse effects from mosquito bites or the ITNs and are free to leave the study at any time. In malaria endemic areas sleepers should be regularly monitored for malaria parasitaemia to ensure that no individual carrying parasites participates in the study and treatment should be provided to any volunteers who become sick with malaria during the study.

7 Considerations for study site selection

7.1 Community study site selection

Community studies need to be conducted in three malaria endemic areas where the ITNs are likely to be sold or distributed, and where ITNs are habitually used by the population. It is preferable to conduct trials in areas where mosquito bites are likely to encourage use of the ITNs, and the selection of the study sites should be justified in the study report.

7.2 Semi-field study site selection

Semi-field studies can only be conducted where the required infrastructure, for example a sufficient number of experimental huts or an IACT, is available, and therefore semi-field studies may be conducted at a site external to the community study(ies) site.

When selecting sites for semi-field studies, manufacturers should consider the composition of mosquito populations, including local species/strain characteristics, in the selection of sites for experimental hut studies, and the necessary characteristics of laboratory strains for use in IACT studies. The vector population (or laboratory strains) at selected sites should exhibit traits in alignment with the defined

primary target(s) based on the mode of action of the AI(s) and intended effects of the product. To assist with study site selection, characterization data for the vector population's target traits, for example, WHO susceptibility tests, insecticide resistance intensity assays, genomic screening, etc., generated by the study site should be considered.

8 Study materials

8.1 Receipt and storage

The means by which study materials, i.e., test and reference items, were obtained, the batch numbers, manufacturing date, the date of receipt, storage conditions prior to distribution, details of storage conditions of sampled nets prior to testing, and the duration of storage prior to distribution and testing should be documented. Data loggers should be used to monitor storage conditions (temperature and relative humidity, at a minimum).

The pre-distribution storage conditions should reflect, in as much as possible, the manufacturer-recommended storage conditions. Any deviations from recommended storage conditions must be documented and presented in the study report. The duration of storage between receipt at the testing facility and the commencement of the study must be documented and included in the report. The median and ranges of the daily temperature and relative humidity of the ITN storage between receipt and distribution should be presented in the study report.

After the study has commenced, it is important to ensure that ITNs that may be distributed at interim points in the study, for example, to replace sampled nets, retain the same characteristics as the original distributed ITNs. Therefore, replacement ITNs (plus an additional number to allow for error) from the same batches used in the study should be stored by the manufacturer at the manufacturer-recommended storage conditions and shipped to the relevant testing facility at the required timepoints (Table 1).

ITNs sampled from study households should be kept in cold storage until testing, and the duration of storage should be minimised in as far as is practicable.

The storage conditions for sampled net pieces should align with the storage requirements for the specific downstream testing for which the sample has been taken. Any deviations from these storage conditions must be documented and presented in the study report.

Table 1. Management of ITN storage and replacement nets for households during long-term community studies

Timepoint	Study sponsor (SS)	Contract Research Organisation (CRO)		
		Distribution and sampling	Chemical/physical characterization	Semi-field study
Pre-distribution	• ITNs shipped from SS to contract research organization			
	• ITNs to be used in household replacement (from the same batches) are stored by SS at recommended storage conditions			
Distribution		ITNs distributed to study households		
6 months post-distribution	ITNs to replace those sampled from households shipped to CRO	ITNs for list B 6 month follow-up surveys sampled from houses and replaced with ITNs received from SS*	List B chemical characterization	
12 months post-distribution	ITNs to replace those sampled from households shipped to CRO	ITNs for list B 12 month follow-up surveys sampled from houses and replaced with ITNs received from SS*	List B chemical/physical characterization	
18 months post-distribution	ITNs to replace those sampled from households shipped to CRO	ITNs for list B 18 month follow-up surveys sampled from houses and replaced with ITNs received from SS*	List B chemical/physical characterization	
24 months post-distribution	ITNs to replace those sampled from households shipped to CRO	ITNs for list B 24 month follow-up surveys sampled from houses and replaced with ITNs received from SS*	List B chemical/physical characterization	
36 months post-distribution	ITNs to replace those sampled from households shipped to CRO	ITNs for list B 36 month follow-up surveys sampled from houses and replaced with ITNs received from SS*	List B chemical/physical characterization	
				ITNs are sampled from houses for use in the semi-field study: • ITNs distributed at the beginning of the study are 36-month operationally aged ITNs • Replacement ITNs that were distributed at 12 months (used in households for 2 years) are 24-month operationally aged ITNs • Replacement ITNs that were distributed at 24 months (used in households for 1 year) are 12-month operationally aged ITNs • ITNs received from the manufacturer at 36-months post-distribution are used as unwashed ITNs

* Sufficient nets should be sampled and replaced at each timepoint to ensure the availability of the required number of ITNs for the eventual semi-field study at the conclusion of the community study.

8.2 Test items

Long-term community studies should be conducted using ITNs from a minimum of three batches. For products that are produced in multiple versions, the selection of the version to be used in the long-term community studies should be based on the submitted pre-market data, particularly the physical tests of the ITN fabric. The version of the ITN that is expected to be the least durable based on these data should be used in the community study. In the development of protocols and designing the study, the use of multiple versions may be considered within or across different studies if scientifically justifiable.

The batches selected for use in community studies should be representative of the production of the product at commercial scale. It is important to note that there should be a reasonable expectation that commercial batches will perform as well as or better than the batches used in the studies.

8.3 Reference items (positive and negative controls)

8.3.1 Negative control (semi-field study only)

The negative control is only required for the semi-field sub-study and when conducting laboratory bioassays. Negative controls should be made of polyethylene or polyester fabric and be free from insecticides.

8.3.2 Considerations for the selection of the positive control

Long-term community studies are conducted with the use of a positive control. The purpose of the positive control is to provide data on site-specific differences in ITN handling/use that may affect fabric integrity and ITN usage data, and to provide a quantifiable signal that can be used to validate experimental procedures in semi-field and supplemental bioassay sub-studies.

The positive control must be an ITN that has the same entomological mode of action and/or combination of AI(s) as the ITN under investigation, and, where possible, be made of the same fabric type, for example, polyester or polyethylene, as the test item. Positive control(s) should be selected and justified based on the intent and design of the study, including the selection of the method(s), endpoint(s), and species/strains, in order to support the assessment of the validity of the study.

Although statistical comparisons between the results for the positive control and the ITN under investigation are used in the data analysis of long-term community studies, these studies are not considered to be comparative in nature. The positive control is present so that any site- specific differences can be disentangled from the performance of the ITN under investigation (test ITN).

8.4 Sampling plans for constructed ITNs

The sampling plans for chemical, physical and bioassays analyses from ITNs used in community studies should be based on the sampling plan used in the generation of Module 3 and 5 pre-market data. Refer to sections below for further details on sampling plans.

9 Study design

9.1 Study type

Long-term community studies are generally conducted as prospective studies. There may be cases where the requirements for the study can be met as part of a mass distribution campaign; in these cases, PQT/VCP recommends that a PQ200 protocol review application be submitted prior to the start of the study.

Long-term community studies should be conducted as either household randomised or cluster randomised controlled trials. Household randomisation may be preferred in situations where there is no risk of unblinding due to differences in the appearance of the test ITN and positive control. Cluster randomised trials may be preferred when the test ITN and positive control look dissimilar, when there is a likelihood of contamination between study arms, or if the ITNs are distributed programmatically and therefore randomisation at the household level is not possible. The selected study design should be presented with an evidence-based justification in the eventual study report.

9.2 Sample sizes

9.2.1 Long-term community studies

The sample size for long-term community studies should be calculated based on the primary outcome of net attrition, with an additional sample size calculation(s) to determine the number of ITNs required for the List B (ITN characterisation) follow up surveys.

Studies should be powered for at least 80% power.

The following assumptions in the sample size calculations should be reported in the study report:

- Average number of ITNs per household and coefficient of variation;
- Anticipated three-year attrition rates;
- Percentage loss to follow up.

The statistical code used for the sample size calculations should be submitted as part of the study report in the format in which it was produced.

The number of ITNs required for the study should be reported using the table format presented in Table 2*.

Table 2. Sample size reporting for long-term community studies

Category	Number
No. of households (or clusters) for longitudinal attrition and fabric integrity monitoring (List A)	
Households for semi-field analysis, chemical content, physical characteristics and supplementary bioassays (List B) plus an equal number to replace sampled nets	
Unadjusted total households (or clusters) per product per study site	
Loss to follow up households	
Adjusted total households per product per study site	
The average number of ITNs per household per product	
Total ITNs per product	
Allowance for error and ITN replacement	
Total ITNs required for each product per study site	

*Note that the same ITNs can be used for the chemical content and physical characteristics if correctly stored to ensure no change in product characteristics after time of sampling.
** Adapted from (3)

9.2.2 Semi-field study

A separate sample size calculation will be required to determine the number of nets required for the semi-field study. Sample sizes are based on the primary endpoint (usually mosquito mortality). A power calculation should be conducted to determine the required sample size and the number of nights of collection that are required.

Semi-field studies using a 9 x 9 Latin Square Design (LSD) should conduct the sample size calculation using simulations based on the primary endpoint, thus simplifying power calculations (refer to section 9.4.5.4.3 for a description of the study arms in the semi-field study). Variability includes the average number of mosquitoes caught per night or released per chamber, per replicate for each species, as well as differences between huts or chambers, between sleepers and between nights. Data from recent trials or pilot studies should be used to parameterize sample size estimations.

The summarised steps to conducting sample size calculations for a semi-field study for which a non-inferiority statistical analysis will be conducted (using the example of mortality as the primary endpoint) are:

i. Estimate mosquito mortality observed for either the positive control, the unwashed test ITN or the 20x washed test ITN
ii. Input the number of mosquitoes caught in experimental huts or released per chamber per species
iii. Estimate the variability between huts or chambers, sleepers and night

iv. For community studies the variability between ITNs should also be included based on previous studies or a conservative estimate of at least 50%
v. Define the number of experimental huts or chambers that will be used
vi. Use data i–iv together with the defined effect margin based on 7% absolute difference in mosquito mortality between the **20x washed test ITN** and the **36-month operationally aged test ITN** to simulate theoretical results for all trial arms (assuming that the percentage mortality follows a binomial distribution). To estimate study power, the true mortality of the **test product** (i.e., the underlying actual probability that a mosquito will die) should be the same as that of the positive control
vii. Fit the logistic regression model to be used for analysis taking into consideration sources of variability to simulated data and determine whether the **20x washed test product** or **36-month operationally aged product** is the same as the reference product
viii. Repeat steps v–vi 1000 times and calculate the percentage of times the test product is the same as the **reference product**. Record this as study power.

If study power is <80% steps v–viii should be repeated, adjusting the number of replicates used (increasing chambers for each trial arm, the number of rotations, the number of mosquitoes or the number of field aged nets evaluated) until the desired power of >80% has been reached.

Statistical codes and tutorials to assist with the determination of sample sizes for semi-field studies and supplementary bioassays can be found at https://github.com/JDChallenger/WHO_NI_Tutorial.

9.3 Controls, Allocation and Distribution

9.3.1 Pre-distribution

9.3.1.1 Baseline quality check of positive control and test items

Prior to distribution, a baseline quality check to characterise the chemical, physical and biological attributes of the ITN fabric(s) using the selected chemical and bioassay test methods should be conducted to identify if any significant changes in the product have occurred during the transport, receipt, storage and handling of fabric samples. The baseline quality check comprises the tests presented in Table 3:

Table 3. Tests conducted during baseline quality checks

Discipline	Tests	Timepoint	Number of samples per fabric type (per net)
		Baseline	
Chemical characteristics	Mean AI/synergist content	x	3
Chemical characteristics	Wash resistance index	x	3
Physical characteristics	Bursting strength (fabric)	x	2
Physical characteristics	Bursting strength (seam)	x	2 for homogenous ITNs, 3 for mosaic ITNs
Entomological characteristics	Bioassay using the selected bioassay method (note that the selected bioassay method should align with that used in pre-market data generation)	x	3

The baseline quality check is conducted on five ITNs from each batch of the ITN under investigation as well as five ITNs from the positive control study arm. Full details of the sampling and conduct of baseline quality checks can be found in the document: Semi-field studies for ITNs: experimental hut and IACT studies.

The baseline quality check should be conducted immediately prior to the distribution of ITNs to study households to ensure that the characteristics of the ITNs measured in the baseline quality check are indicative of the distributed ITNs when they arrive in study households.

In the final part of the baseline quality check, a visual inspection of 3 ITNs per bale received is conducted to ensure that the products to be used in the study conform to the expected appearance. When selecting ITNs from each bale, each ITN should be sampled from a different position, for example, top of the bale, middle of the bale, bottom of the bale. The sampling strategy for the selection of ITNs from bales should be presented in the study report.

The inspected parameters for the visual inspection are:

- Fabric integrity, including seams;
- Colour;
- Symmetry;
- Material.

Any indications that the ITNs for use in the study do not comply with the expected standard, for example, discolouration, unravelling seams, etc. should be recorded and presented in the study report. Damaged ITNs that do not conform with the expected visual appearance should not be distributed.

9.3.1.2 ITN labelling

Each study ITN is labelled with a unique identifier to allow the ITN to be tracked within the study if they are not labelled with GS1-Coding system.

9.3.2 Distribution

9.3.2.1 Baseline surveys and distribution of ITNs

After community sensitisation, the baseline survey and distribution of ITNs may be conducted on the same day as study enrolment. If conducting the baseline survey and ITN distribution on the same day is not logistically feasible, this stage may be conducted over multiple days.

Informed written consent should first be obtained from the head of household or primary resident. A household is defined as "people eating from the same pot".

The baseline survey should be conducted by trained study personnel who then distribute the ITNs required for the household. One ITN is distributed per sleeping space. A sleeping space is defined as any bed, mattress, mat or other indoor sleeping surface where people regularly sleep (4).

Criteria for ITN distribution to sleeping spaces are:

- Regular use: the space must be used regularly for sleeping;
- Accessibility for net hanging: the space should be suitable for hanging or installing an ITN to ensure full coverage and protection;
- Number of sleepers: Consider the number of people who sleep in the space to determine the number of nets that are required.

Non-study ITNs already present in the household are withdrawn to ensure that only ITNs from the study are present and used in households. Handling of withdrawn nets, i.e. whether the nets are stored and returned at the conclusion of the study or discarded, should be conducted according to the ethical considerations and regulations for the country in which the study is conducted.

The unique identifier of the ITN is recorded in the baseline questionnaire and the ITN master list. ITNs within a household may be allocated to the A master list or the B master list (see below) but not to both.

Where feasible, it is recommended that information on the indoor environmental conditions, such as temperature and humidity, be collected through the deployment of data loggers.

9.3.2.2 Randomisation to study arms

9.3.2.2.1 ITN distribution

Following the baseline survey, an ITN product is assigned to the study household or households within a cluster using a suitable method, for example, a lottery method. An example of conducting a lottery method for a household randomised study is as follows:

- Each member of the study personnel conducting the baseline surveys is given a bag containing products from every arm in the study;

- At each household, a product is selected at random from the bag, and, once the product is selected, additional ITNs of the same product are distributed to each sleeping space in the household.

For a cluster-randomized study, clusters and study households should ideally be assigned using a suitable randomization method, such as a random number generator. If the assignment is not random, for instance, if the study is conducted as part of a program, it is crucial to justify the steps taken to ensure that the selection of clusters and households within clusters does not introduce bias into the study.

9.3.2.2.2 Generation of master lists for follow up surveys

The ITN master list is compiled from the ITNs distributed to study households and contains:

- ITN unique identifier;
- Household identifying code;
- Household GPS coordinates (name of village and name(s) of head(s) of household can be used in place of GPS coordinates if indicated).

To generate the master lists for follow up surveys from the ITN master list, a random number generator is used to create two lists of households:

- Attrition, fabric integrity monitoring, ITN survival and user acceptability **(List A);**
- Efficacy and characterisation of ITN fabric (chemical content, physical characteristics and laboratory bioassays) **(List B).**

ITNs on List A are followed until the end of the survey (or until the household is lost to follow up or withdraws consent) while ITNs on List B are removed from the household for destructive sampling. New ITNs are provided to the household when ITNs on List B are removed.

9.3.2.3 Adverse events

Prior to the commencement of the study, events that constitute serious adverse events must be defined for the product in consultation with the study sponsor. Within 30 days of the commencement of the study, any serious adverse events experienced by study participants must be reported to the study co-ordinators by study participants using the contact information provided in the study consent form.

No more than 10% of respondents should experience serious adverse events. If the 10% criterion is breached, the study must be halted and the ITNs withdrawn from all study households while an investigation is conducted.

9.4 Household surveys

Household surveys are conducted at ITN distribution and during the defined follow up timepoints in the List A and List B households (Table 4).

The gender of respondents is collected during surveys and the gender breakdown of respondents must be presented in the study report. If the gender balance is significantly skewed, a justification must be provided in the report.

Table 4. Household surveys in long-term community studies

	Survey	Study timepoint					
		Baseline/ITN distribution	Follow up surveys				
			6	12	18	24	36
	Baseline survey	X					
	ITN baseline net listing and sampling	X					
	User acceptability survey					X	
List A	Attrition		X	X		X	X
	Fabric integrity		X	X		X	X
List B	Follow up sampling survey		X	X	X	X	X

9.4.1 Baseline survey

The baseline survey can be conducted at the same time as the distribution of ITNs to study households. Survey questionnaire forms should be written in local languages and ideally survey forms are pre-tested and either pre-loaded onto electronic data collection devices or printed as paper record forms prior to the commencement of the survey.

Data collected during baseline surveys are used for:

- The household roster (names, ages, sex, education, occupation, relationship to household head);
- Household wealth index;
- House characteristics (roof, wall and floor material);
- Number of sleeping spaces;
- Characteristics of sleeping spaces (floor, mat, unimproved or improved bed);
- Ownership of mosquito nets;
- Use of mosquito nets;
- Net attitude indicators;
- GPS coordinates of household.

Considering the nature of the information collected, appropriate procedures must be implemented to ensure the collection, storage and use of the information conforms with relevant laws and regulations pertaining to study participants privacy.

Suggested baseline surveys are available in Annex I.

9.4.2 User acceptability survey

User acceptability surveys are conducted to collect data on community preferences and practices which may influence the use of an ITN in a specific setting. The user acceptability survey is conducted in all households that are surveyed for net attrition.

Data collected during the user acceptability surveys are:

- User preferences.

9.4.3 Follow up surveys (List A)

Follow up surveys are conducted at 6, 12, 24, and 36 months after distribution. All households that are assigned to List A are surveyed at each timepoint.

Suggested follow up survey questionnaires for List A monitoring are available in Annex I. To aid in ease of use, the questions for attrition and fabric integrity monitoring are presented in a single survey form. The relevant sections of the survey form are indicated in the appropriate sections below.

9.4.3.1 Attrition

Attrition monitoring is conducted to determine the proportion of ITNs no longer found in their respective households. The primary data point collected in attrition monitoring is the presence or absence of the study ITN(s) in the household.

During the attrition survey, the presence or absence of all the ITNs that were distributed is recorded. If ITNs are absent, the reasons for absence are provided by the head of the household.

- ITNs missing due to wear and tear, as well as those that have been repurposed due to damage are considered to be **lost to attrition**.
- ITNs missing due to being given away, that are lost, stolen or that are being used elsewhere are considered to be **lost to follow up**.

9.4.3.1.1 Survey timepoints

Attrition monitoring is conducted at 6, 12, 24 and 36 months post-distribution.

9.4.3.1.2 Study acceptability criteria associated with this sub-study

If the proportion of ITNs lost to attrition in the test arm is greater than 50% at any timepoint, the study is halted.

9.4.3.2 Fabric Integrity

Fabric integrity monitoring is conducted to determine the physical state of ITNs that are in use and found within study households during follow up surveys. The indicators of interest in fabric integrity surveys are the proportion of ITNs with holes, and the extent of damage to the ITNs measured by the holed area or the proportional hole index.

Each in use study ITN that is found within a household during the attrition monitoring is draped over a collapsible net frame to facilitate the locating and counting of all holes that are present (Fig 3). The collapsible frame should be the same size as the study ITNs.

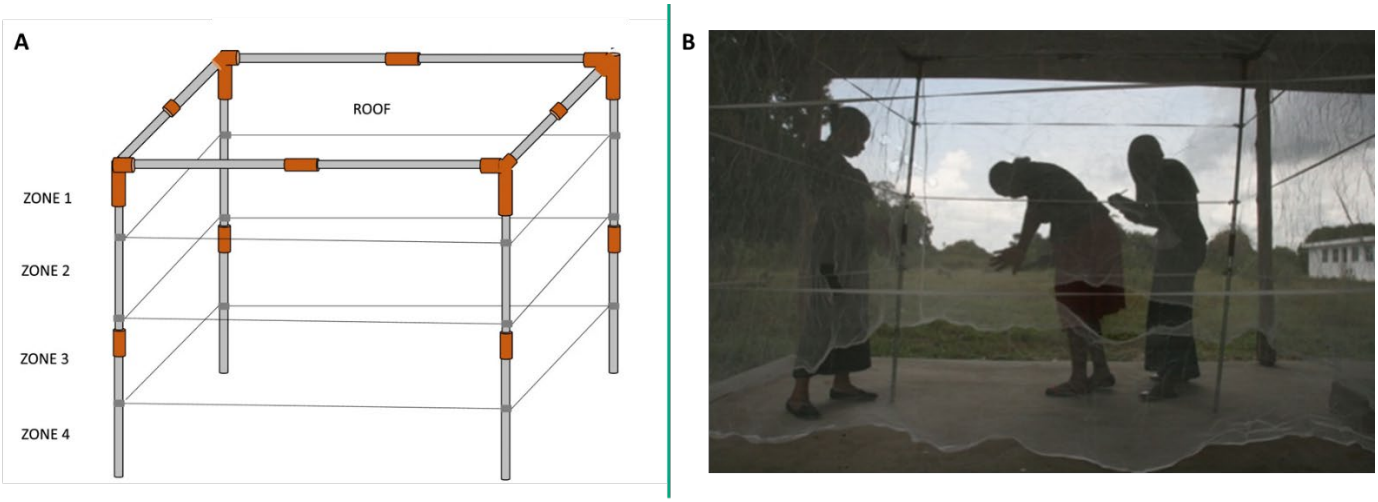


Figure 3. Design for collapsible net frame with zones for hole counting used for fabric integrity monitoring (A); net frame for fabric integrity monitoring in use (B)

Credit: A: Adapted from Lorenz et al, 2014 (ref). B: Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, 2014.

9.4.3.2.1 Survey timepoints

Fabric integrity monitoring is conducted at 6, 12, 24 and 36 months post-distribution.

9.4.3.2.2 Counting and classification of holes

Holes in ITNs are counted and located in five zones. Once draped over the collapsible frame, ITNs are divided into four equal zones from the top (zone 1) to the bottom (zone 4) of the net. The roof panel comprises the fifth zone. The zonal system allows for the location of any holes present in the net to be recorded more accurately.

Holes are classified into four size categories (Table 5):

Table 5. Net hole size classification

Category	Original category description	Hole size (cm diameter)
Size 1	Smaller than a thumb	0.5 – 2
Size 2	Larger than a thumb but smaller than a fist	2 - 10
Size 3	Larger than a fist but smaller than a head	10 – 25
Size 4	Larger than a head	>25

Tears and split seams should be counted as holes.

Holes smaller than 0.5 cm should be ignored.

The size and location of holes in each ITN is entered in the hole tally sheet and entered in the follow up questionnaire (Annex I). If repairs to holes are noted during inspection, this information, including the type of repair, is also collected as part of the survey. Repaired holes are not counted as holes.

Visual aids to assist with the classification and approximation of net hole sizes and training materials for field teams have been developed with the support of the U.S. President's Malaria Initiative and can be located here:

- Field tool for tallying and hole size
  - » https://www.durabilitymonitoring.org/wp-content/uploads/2015/10/3c.-Two-page-Field-Tool-for-Tallying-and-Hole-Size.pdf;
- Training slides for hole assessment
  - » https://www.durabilitymonitoring.org/wp-content/uploads/2015/10/3b.vi_.-LLIN-Hole-Assessment-Slides.pptx;
- Visual aid to classify hole type:
  - » https://www.durabilitymonitoring.org/wp-content/uploads/2015/03/3c.iii_.-Visual-Aid-for-Types-of-Holes.pdf

9.4.3.3 Functional survival

The functional survival of ITNs is an estimation of the proportion of nets that remain in use in households in a serviceable condition. It is calculated using the results from attrition and fabric integrity surveys. Refer to section 10.2.2.1.1 for details.

9.4.4 Follow up surveys (List B)

List B follow up surveys are conducted at 6, 12, 18, 24 and 36 months post-distribution

9.4.4.1 Chemical and physical analyses

Chemical analyses of sampled net pieces are conducted to quantify the total AI content and surface AI content of operationally aged ITNs collected from study households and are conducted at specific timepoints during the community study.

Physical inspection and analyses are conducted to assess the physical integrity of the distributed ITNs, and to assess changes in physical parameters over the duration of the community study.

Note: In the WHO guideline for prequalification assessment of ITNs, a list of physical tests was included in section 7.5. In the development of this implementation guidance, it was determined that the physical tests abrasion resistance, snag strength, and resistance to hole formation, would not provide additional value to the study results. These methods were developed for the purpose of informing pre-market predictions of the physical durability. As the long-term community study includes tests for fabric integrity, for example, proportional hole index, there is the opportunity to better correlate and investigate the relationship between these predicted values and the hole formation data from operational use. Bursting strength was retained as a measurement within the study so as to enable identification of any significant degradation in integral component/fabric durability during operational use. Study sponsors and their partners may choose to include other physical tests within their study design. In such cases, the purpose and use of the collected data should be considered.

9.4.4.1.1 Considerations for chemistry method selection

In chemical analysis, the total AI of sampled fabric pieces should be measured using the reference enforcement analytical method or validated in-house method supported by an analytical bridging study to the reference method.

9.4.4.1.2 Sampling timepoints

Chemical and physical analyses are conducted:

- As part of baseline quality checks prior to the study commencing;
- At 6, 12, 18, 24, and 36 months post-distribution.

The chemical and physical analyses conducted at each timepoint are presented in Table 6:

Table 6. Chemical analyses in long-term community studies

Test	Timepoint (months)					
	Baseline	6	12	18	24	36
Appearance/description*	x	x	x	x	x	x
Mean AI/synergist content	x	x	x	x	x	x
Mean surface AI/synergist content		x	x	x	x	x
Wash resistance index	x					x
Bursting strength (fabric and seam)	x		x		x	x

* For those ITNs sampled for List B activities, a qualitative description should be collected to denote the physical state/appearance

Results for each parameter should be assessed separately for each batch at baseline. At all subsequent timepoints sampling should be batch agnostic as batch identification may not be feasible.

It is recommended that the chemical analyses be conducted at the time of sampling and therefore that samples are not held for analysis at the conclusion of the study. This is because the chemical analysis data may be useful in identifying any unexpected changes or declines in AI reservoir that may require investigation and early termination of the study.

The minimum number of ITNs to be collected for physical/chemical analyses at each timepoint and the number of samples to be taken from each ITN fabric for each attribute are shown in Tables 7 and 8:

Table 7. Number of ITNs to be sampled from households for physical/chemical testing during long-term community studies

Parameter	Timepoint (months)				
	6	12	18	24	36
Number of ITNs to be sampled for List B testing requirements (as per Table 6)*	30	30	40	50	50

* Note: for the bursting strength test, samples need only be taken and tested from 25 of the total ITNs collected per time point.

Table 8. Samples for chemical analysis*

Test	Samples per ITN fabric (n)	Sample size (area)	Method
Mean AI/synergist content	3	The size of samples (for example, 25 × 25 cm) should be selected to comply with the selected method for AI quantification and to provide results with a relative standard deviation (RSD) ≤ 5% or as applicable in justifiable cases.	The analytical method should be selected based on the AI and applicable validated method. Method(s) of analysis must be CIPAC, AOAC or equivalent. Where methods have not yet been published, full details and appropriate method validation data must be submitted.
Mean surface AI/synergist content	3	The size of samples (for example 25 × 25 cm) should be selected to comply with the selected method for AI quantification and to provide results with a relative standard deviation (RSD) ≤ 5% or as applicable in justifiable cases.	The analytical method should be selected based on the AI and applicable validated method. Method(s) of analysis must be CIPAC, AOAC or equivalent. Where methods have not yet been published, full details and appropriate method validation data must be submitted.
Wash resistance index	3	25 × 25 cm pieces in accordance with the IG – Determination of wash resistance index test for ITN sampling plan	Adapted CIPAC (O) MT 195 - IG – Determination of wash resistance index test for ITN fabric
Bursting strength (fabric)	2	Tests of 7.3 cm^2 areas of fabric	ISO 13938 part 2: 2019 with conditioning of the fabric as specified in the ISO standard.

Test	Samples per ITN fabric (n)	Sample size (area)	Method
			If physical damage is present, samples must be taken from undamaged areas of the fabric.
Bursting strength (seam)	For homogeneous ITNs: 2 samples per ITN Sample 1: side-side Sample 2: side-roof For mosaic ITNs: 3 samples per ITN Sample 1: side-side Sample 2: side-roof Sample 3: side-roof	Tests of 7.3 cm^2 areas of fabric Samples should be taken in a manner which allows for the seam to be centered on the test head.	ISO 13938 part 2: 2019 with conditioning of the fabric as specified in the ISO standard If physical damage is present, samples must be taken from undamaged areas of the fabric/seams.

*Samples and sizes may need to be augmented for products which contain multiple active ingredients.

Fabric samples which are not immediately analysed for chemical content should be individually wrapped in aluminium foil and held at 4°C.

Refer to the wash resistance index implementation guidance document for details of the test method.

Further details of sampling for chemical characteristics can be found in the implementation guidance document Declaration of ITN construction and sampling.

9.4.5 Entomological analyses

Two types of entomological analyses are conducted during community studies:

- Laboratory bioassays are conducted as part of the baseline quality check to confirm the characteristics of the distributed product.
- Semi-field assessments are conducted to characterise the entomological efficacy of sampled operationally aged ITNs. ITNs are sampled at specific timepoints during the community study and the entomological efficacy is assessed at the end of the community study.
    - » Supplementary bioassays to ensure the consistency of the ITNs used in the semi-field study are conducted alongside the semi-field study.

9.4.5.1 Sampling timepoints

Bioassays are conducted:

- As part of baseline quality checks prior to the study commencing.

The semi-field analyses are conducted with all arms evaluated concurrently:

- Using unwashed, unused ITNs;
- Using sampled ITNs that have been in operational use for 12, 24 and 36 months;
- Using ITNs that have been washed 20 times to allow correlation between submitted pre-market data and operational use.

9.4.5.2 Selection of entomological endpoints

The potential endpoint(s) which may be selected for use in a long-term community study, the accompanying semi-field study and the supplementary bioassay(s) must be representative of the intended effect of the product. The selection of appropriate endpoint(s) may dictate the selection of the method and/or encourage the use of multiple entomological methods.

The endpoint(s) selected for use in long-term community studies must be the same endpoint(s) as those used for data generation in Module 3 and 5 entomological studies.

9.4.5.3 Considerations for entomology method selection

9.4.5.3.1 Supplementary bioassays and bioassays for baseline quality checks

Typically, long-term community studies use the cone test or the tunnel test as the bioassay method for supplementary experiments, based on the mode of action of the AI(s), the intent of the product, and the bioassay method used for data generation in module 3 and 5 studies. Closed system free-flying mosquito bioassays, for example, an ambient chamber test (IACT), can be considered as a substitute for tunnel tests.

Other existing or novel methods can be proposed in situations where the standard methods are not appropriate, if those methods have been used in the data generation for Module 3 and 5 studies. If another method is being considered or augmentations to standard methods are necessary, WHO recommends that substantiating documentation be provided with a protocol review request submission.

A single bioassay method should be selected for use in supplemental bioassays, except if there is a need to use multiple bioassays to demonstrate the intended effect of multiple AIs.

9.4.5.3.2 Considerations for test system species/strain selection

For the purposes of a long-term community study, the selected test systems should be relevant to the intended use of the product, i.e., vectors of the disease(s) intended to be impacted. The selected strains should be characterized in terms of the susceptibility to the AI(s) and the specific mechanisms of resistance, if applicable. The use of multiple species/strains in supplemental bioassays in a long-term community study can provide valuable information about:

- the differences in time until effects are observed in relation to species/strain characteristics;
- identification of the potential range of response (baseline) for selected endpoints measured in the bioassay in relation to species/strains.

Where multiple test system species/strains are used, the test system species/strain that will be used to determine whether the product has demonstrated the required characteristics must be clearly identified and justified.

9.4.5.4 Semi-field studies

9.4.5.4.1 Local vector population characteristics in semi-field site selection

Manufacturers should consider the composition of mosquito populations, including local species/strain characteristics, in the selection of sites for the semi-field component of long-term community studies. The vector population at selected sites should exhibit traits in alignment with the defined primary target(s) based on the mode of action of the AI(s) and intended effects of the product. To assist with study site selection, characterisation data for the vector population's target traits, for example, WHO susceptibility tests, insecticide resistance intensity assays, genomic screening, *etc.,* generated by the study site should be considered.

Additionally, manufacturers should consider the National Regulatory Authority requirements for product registration in order to prioritize generation of efficacy data which can be used to support registration and/or selection decisions across multiple countries/organizations.

9.4.5.4.2 Considerations for method selection for semi-field analyses

The semi-field study conducted at the conclusion of the community study, i.e., after 36 months of operational use of ITNs, can be conducted using any semi-field method that is currently recognised by WHO.

9.4.5.4.3 Study arms for semi-field analyses

The semi-field study should be conducted according to the methodology described in the IACT or experimental hut method implementation guidance document and the semi-field studies implementation guidance document, using the following minimum treatment arms in a 9x9 LSD:

1. Unwashed net (negative control)
2. Unwashed Test ITN
3. 20x washed Test ITN
4. 12-months old Test ITN
5. 24-months old Test ITN
6. 36-months old Test ITN
7. Unwashed positive control
8. 20x washed positive control
9. 36-months old positive control

For positive and negative controls, the number of ITNs prepared for each treatment arm should equal the number of huts used in the study plus two to be used in supplementary chemical and biological characterisation.

For both IACT and Experimental hut 9-arm studies, 30 individual ITNs should be collected for use in each arm of the study. To ensure that sufficient ITNs are available for the semi-field study at the conclusion of the community study, it is recommended that at least 38 ITNs for each arm be collected and replaced during follow up surveys.

To power semi-field studies, it is crucial to be aware of the between-net heterogeneity in performance of field-aged ITNs, which will increase the level of variability present in the data. Simulation-based methods have been used to generate the guidelines outlined below. To analyse the observed mosquito mortality data, logistic regression models should be fitted to the data, and should include fixed effects for trial arm, sleeper, and study day (for EHTs an additional fixed effect for hut should be included).

In the case of the IACT, table 9 provides guidance for the duration of the study, assuming an 18-compartment IACT is carried out. The numbers presented in the table use conservative values for the between-ITN heterogeneity. An interim assessment of this heterogeneity could be made during the study. This could allow for a shorter study to be performed, if the heterogeneity is found to be lower.

Table 9. IACT semi-field study durations

Number of mosquitoes released per IACT chamber per night	Duration of study (days)	Data points generated per individual ITN (30 ITNs per arm used in study)
15	68	4.5 (i.e. some ITNs used 4 times, some 5 times)
20	60	4
25	52	3.5

In the case of the EHT, there is additional uncertainty, due to the fact that wild, free-flying mosquitoes are used, the densities of which cannot be controlled by the investigators. Following analysis carried out on data from previous hut trials (5) these are sampled from a negative binomial distribution, where the mean of the distribution is varied. The measured, or anticipated, mosquito densities in the study area should be used to determine the duration of the EHT, as outlined in table 10. For sites with high mosquito densities, it will be sufficient to perform a nine-hut trial (with the 9 x 9 LSD outlined above), for a single rotation, generating 81 data points for each arm. In areas with relatively low mosquito densities (fewer than ten mosquitoes per hut per night, on average), a twelve-hut study could be used, in which the three arms with the operationally-aged test ITNs are used in two huts simultaneously, thereby doubling the number of data points generated for these study arms.

Table 10. Experimental hut semi-field study durations

Mean number of mosquitoes per hut per night	Number of huts to use	Number of rotations of the LSD to conduct	Number of days required for the study (ignoring rest days)	Replicates per individual ITN
5	12 (with two huts in use for each of the three arms with operationally-aged ITNs)	1	144	9.6 for operationally-aged ITNs; 4.8 otherwise
10	9	1.5	121	4.05
15	9	1	81	2.7
20	9	1	81	2.7

9.4.5.4.4 Baseline checks prior to semi-field studies

If the semi-field study conducted is an experimental hut study, baseline information regarding the attractiveness of the huts and the recapture rates of mosquitoes released in the huts, i.e., hut retention, should be collected prior to the semi-field study commencing. The scavenging rate should be estimated by placing dead mosquitoes in petri dishes in huts overnight. The baseline period serves to re-train staff and increase the attractiveness of the huts to mosquitoes through indoor sleepers. The environmental conditions in experimental huts (temperature and humidity) should be continuously monitored with a data logger.

Experimental hut semi-field studies should only be conducted during times when mosquitoes are abundant.

For further details on the method and conduct of semi-field studies, refer to Semi field studies for ITN: experimental hut and IACT studies.

10 Results and Data analysis

Results for test samples and reference items (negative and positive controls) should be presented in both tabular and graphical format.

If ITNs constructed from multiple fabrics have been used in the study, results for the supplementary bioassays must present the results for each fabric separately, for example, results for the roof and sides

for a mosaic net where the roof and sides have been constructed from different fabrics should be presented as [Product A roof] and [Product A sides].

Descriptive and inferential statistics with appropriate error measurements should be used to present results.

10.1 Pre-distribution

10.1.1 Baseline quality check – chemical analysis

The results to be reported for baseline chemical quality checks are:

- Arithmetic mean results with respective standard deviation;
- Percentage Relative standard deviation (RSD).

The inter- and intra-batch variability are analysed using RSD to measure the precision. RSD should be expressed as percentage. It is obtained by multiplying the standard deviation (SD) by 100 and dividing by product average (%RSD = SD *100/Mean).

A table showing the summary results (number of net pieces, mean concentration of AI, SD, range, %RSD) per net, production batch and overall should be included in the report.

Suggested table formats are presented in Annex II.

10.1.2 Baseline quality check – bioassays

The results to be reported for baseline quality bioassays are:

- Arithmetic mean results with 95% CIs for each selected endpoint.

A table showing the summary results (number of mosquitoes exposed, number of replicates, percentage arithmetic mean and 95% confidence intervals) per net, per production batch and overall should be included in the report.

Suggested table formats are presented in Annex II.

10.1.3 Baseline quality check – visual inspection

Present the sampling strategy for the selection of ITNs from bales. Present the results of the visual inspections in tabular format.

Suggested table formats are presented in Annex II.

10.2 Post-distribution

10.2.1 List A surveys

In addition to product characteristics, the environment in which an ITN is used and the behaviour of the user(s) will affect the ITN median functional survival. The results from the list A surveys are therefore of use in supporting the interpretation of study results.

10.2.1.1 Summarised study characteristics

The total number of households enrolled, number of study ITNs distributed, number of ITNs followed per timepoint should be presented in the study report in tabular form, as should the consent results from each survey round.

Suggested table formats are presented in Annex II.

10.2.1.2 Net attitude score

The net care mean attitude score is based on eight questions that measure beliefs about the effectiveness of net care and repair, the value of ITNs, whether it is a social norm, and one's ability to practice net care and repair (questions 68 – 75 in the Baseline and List A follow up surveys) (6). The answers have a four-value response, omitting the neutral option, from strongly agree (+2) to strongly disagree (-2). The net attitude score is calculated using the answers to questions 68 – 71 and 73 – 75.

To calculate the net attitude score:

- Transform the answers to questions 70 and 73 by multiplying by -1;
- Add the scores of questions 68 – 71 and 73 – 75, taking care to use the transformed scores for questions 70 and 73;
- Obtain the average score by dividing by 7.

The net attitude score should be presented in the study report as an aggregate and as separate scores for the baseline, 6, 12, 24 and 36 months timepoints, in tabular format. Additionally, the net attitude score(s) may also be used as covariates in the functional survival analysis; if this is done, then full methodological details must be presented.

10.2.1.3 User acceptability survey

The results to be reported for the user acceptability survey are:

- List of preferences and ranks for preference;
- Descriptive statistics of user preference.

User preference may vary within and between households and across geographies. Summaries can be presented as a percentage of the sampled population. Ensure that the gender breakdown of respondents is included in the presentation of results.

10.2.1.4 Fabric integrity

10.2.1.4.1 Proportion of ITNs with holes

To calculate the proportion of ITNs with any holes, the numerator is the total number of each ITN product with at least one hole of sizes 1-4, and the denominator is the total number of each ITN product found and assessed in survey households.

This indicator, along with the number of ITNs and percentage of ITNs with holes, should be calculated and presented for each category of hole size.

10.2.1.4.2 Hole area and proportionate hole index

The holed area of an ITN is an estimate calculated by categorising the holes into four categories based on whether a finger, thumb, fist or head can pass through or by directly measuring hole diameter. Data should be entered either with actual measured values of hole diameter or by hole category (1, 2, 3 or 4, Table 11).

Hole categories are used to calculate the proportionate hole index and is based on an assumption that the holes in each size category are circular, with a diameter that is equal to the mid-point of the category (excepting the largest category for which a diameter of 30 is selected). The area of the hole is then calculated using $A = \pi r^2$, where $\pi = 3.142$ and $r =$ the diameter divided by 2 and summed over each net.

Table 11. ITN hole size and hole size categories

Hole size category	Hole size (cm)	Hole diameter (d; cm)	Hole radius (r = d/2)	r^2	Area of hole (πr^2)	Hole index[a]
1	0.5 – 2.0	1.25	0.625	0.390625	1.23	1
2	2 – 10	6	3	9	28.28	23
3	10 – 25	17.5	8.75	76.5625	240.56	196
4	>25	30[b]	15	225	706.95	576

A, area of the hole; $\pi = 3.142$; [a]Area divided by 1.23; [b]Assumed diameter

The overall physical condition of the net is obtained by weighting the number of holes of each size by 1, 23, 196 and 596 for the four hole size categories to calculate a proportional hole index (pHI). The equation to calculate the pHI is:

$$pHI = (A \times no.of\ size\ 1\ holes) + (B \times no.of\ size\ 2\ holes) + (C \times no.of\ size\ 3\ holes) + (D \times no.of\ size\ 4\ holes)$$

where A = the weight of size 1 holes (1), B = the weight of size 2 holes, C = the weight of size 3 holes and D = the weight of size 4 holes.

For each product type, the median and interquartile range for either the hole area or the proportionate hold index should be calculated and presented.

10.2.1.4.3 Interpretation of results

If hole area is used: ITNs are classified as serviceable if the hole area is less or equal to 789cm and too torn if the hole area is equal to or greater than 790cm if the holes are assumed to be circular.

If the proportionate hole index is used: ITNs are classified as serviceable if the pHI is less or equal to 642 and too torn if the pHI value is equal to or greater than 643.

10.2.2 Functional survival of ITNs

The functional survival of ITNs is calculated using results from attrition and fabric integrity surveys using the formula:

$$Functional\ survival = \frac{Number\ of\ nets\ present\ and\ serviceable}{Number\ of\ nets\ originally\ received\ and\ that\ have\ been\ used\ for\ sleeping\ under\ and\ not\ given\ away, lost\ to\ follow\ up}$$

10.2.2.1 Median survival time and median functional survival time

10.2.2.1.1 Median survival time

The median net survival in years is the timepoint at which the estimate of functional survival of an ITN product crosses 50% (7). To determine the median survival time, a S-shaped curve is first fitted to the functional survival data (using the equation given in Section 10.2.2 measured at each timepoint. The following functional form is used (8-11):

$$f(k, L, t) = \exp\left(k - \frac{k}{1 - \left(\frac{t}{L}\right)^2}\right).$$

Here, *L* and *k* are parameters and t is the time since the ITNs were distributed. The function *f(k,L,t)*, which is valid for t<L, gives the functional survival of the ITNs at time *t*. To aid parameter identifiability, (8-11) is followed, k is fixed to a value of 20. A Bayesian modelling framework is used to estimate the value of *L*, which is given a uniform prior distribution U(5.5,20.7) (8-11).

To determine the median survival time (t_{50}), set $f(k, L, t_{50})$=0.5, and rearrange to find t_{50}. In general:

$$t_{50} = L\sqrt{\frac{\ln(2)}{\ln(2) + k}}.$$

Here 'ln' is the natural logarithm and, as above, k is set to a value of 20. Using posterior samples from the Bayesian model, the uncertainty in L can be propagated through the equation, to reflect the uncertainty in t_{50}. For a sample size of 300 households (assuming at least one ITN per household), and assuming a loss rate (over the course of 3 years) of 20%, simulations suggest that t_{50} can be measured with a precision of approximately 60 days, defining precision as the width of the 95% credible interval of t_{50}. Table 12 shows the data that should be recorded-- 6, 12, 24, and 36 months after the ITNs were distributed.

Table 12. Presentation of median functional survival

Timepoint	Time in years	Number of ITNs present and serviceable	Number of ITNs originally received and not given away or lost to follow up	Functional survival (percentage, [95% CI])
6 months	0.5			
12 months	1			
24 months	2			
36 months	3			

10.2.3 List B surveys

10.2.3.1 Chemical analyses

10.2.3.1.1 Total AI/synergist content

The results to be reported for total AI/synergist content are:

- Arithmetic mean results for each sampling time with RSD
- AI/synergist retentions at each sampling time calculated as the mean total content result for that sampling time divided by the mean result at baseline and expressed as a percentage

The AI/synergist retention profile(s) over time should be compared with the results obtained in the laboratory wash and pre-market semi-field studies. Potential causes of any differences should be reported and their implications for the efficacy of commercial batches of the ITN should be discussed in the report.

10.2.3.1.2 Wash resistance index

Report the WRI to the nearest 0.1% and specify the following parameters:

- temperature at which the heating procedure was carried out if different from the standard (40 ± 2°C);
- analytical method used.

Data should not be aggregated for analysis across batches at baseline and the results should be reported in combination as arithmetic means with respective standard deviation for each sampling time at all subsequent timepoints.

Any increasing or decreasing trends over time should be identified and discussed in the report. Note: Some variability is to be expected and does not need to be discussed unless it is greater than would be expected given the results obtained in the pre-market studies.

Refer to the wash resistance index implementation guidance document for further details.

10.2.3.2 Physical analyses

10.2.3.2.1 Appearance/description

The number and percentage of nets examined that comply with the clause for appearance in the manufacturing release specification should be reported for each timepoint. Any differences with the manufacturing release specification should be identified and their implications for the durability of commercial batches of the ITN should be discussed in the report.

10.2.3.2.2 Fabric and seam bursting strength

The results to be reported for bursting strength are:

- Arithmetic means for the fabric(s) and seam(s) at each sampling time;
- The percentage of results for the seam(s) at each sampling time that are greater than the result for at least one of the adjoining fabrics.

Any decreasing trends should be identified and their implications for the durability of commercial batches of the ITN should be discussed in the report.

10.2.3.3 Semi-field studies

To ensure standardization of analytical approaches, a specific model must be used when performing the analysis and presenting the results for assessment. To relate the outcome variables to the intervention and covariates, generalized linear regression models (GLMs) should be used. The choice of model will depend on the endpoint(s) under investigation. For binary endpoints, such as the proportion of mosquitoes dying or the proportion fertile, a logistic model is appropriate. For outcomes that are counts, such as the number of eggs laid, a Poisson or negative binomial model may be more appropriate.

It is recommended that all covariates should be categorical fixed effects and the 20x washed ITN of the test ITN should be used as the reference intervention (intercept). For semi-field studies (experimental hut or IACT), covariates include **net product and preparation**, hut or chambers, sleepers or host and night as fixed effects because these factors are sources of systematic variation that are accounted for in the experimental design.

Net condition (good, damaged or too torn) should be added as a covariate as net integrity influences net entomological efficacy.

Statistical code and tutorials to assist with the analysis of semi-field data and supplementary bioassays can be found at: https://github.com/JDChallenger/WHO_NI_Tutorial

10.2.3.3.1 Criteria for study validity and acceptance

The test ITN must fulfil the following criteria in the semi-field test in order for the study to be accepted:

1. Study power:
    - » The study must demonstrate sufficient power for decision-making, based on the conducted power calculation and the sample sizes of mosquitoes collected during the study (experimental hut studies), ***or***
    - » The study must demonstrate sufficient power for decision-making, based on the conducted power calculation and the numbers of mosquitoes released and replications (IACT)
2. Results for free-flying mosquitoes:
    - » The results for the 36-month operationally aged candidate ITN at the selected endpoint must be statistically significantly higher than the results for the negative control, ***and***
    - » The results for the 36-month operationally aged candidate ITN at the selected endpoint should demonstrate:
        - Non-inferiority of the 36-month operationally aged ITN to the 20x washed ITN of the same product type.

10.2.3.3.2 Supplementary bioassays to semi-field studies

1. Bioassay results for the selected endpoint for the ITN must not be less than 7% lower than the results for the 20x washed ITN, using the odds ratio.

10.2.3.3.3 Characterisation of mosquito strains used in bioassays and local vector population(s) at semi-field sites

Characterisation results for mosquito strains used in bioassays and the local vector population at semi-field sites should be reported using the Matrix of selected mosquito strains template.

11 Related documents

- WHO PQT/VCP Implementation Guidance – Wash resistance index test for ITN fabrics
- WHO PQT/VCP Implementation Guidance – Real-time and accelerated storage stability studies
- WHO PQT/VCP Implementation Guidance – Declaration of ITN construction and sampling procedure
- WHO PQT/VCP Implementation Guidance – Considerations for the selection of controls
- WHO PQT/VCP Implementation Guidance – Considerations for the selection of mosquito strains for use in bioassays and site selection for semi-field and community studies
- WHO PQT/VCP Implementation Guidance – Matrix of selected mosquito strains
- WHO PQT/VCP Implementation Guidance – Matrix of selected mosquito strains template
- WHO PQT/VCP Implementation Guidance – Bioassay methods for insecticide-treated nets: Cone test
- WHO PQT/VCP Implementation Guidance - Bioassay methods for insecticide-treated nets: Tunnel test
- WHO PQT/VCP Implementation Guidance – Bioassay and semi-field methods for insecticide-treated nets: Ifakara Ambient Chamber Test (IACT)
- WHO PQT/VCP Implementation Guidance – Semi-field methods for ITNs: Experimental hut tests
- WHO PQT/VCP Implementation Guidance – Semi-field studies for ITNs: experimental hit and IACT studies
- WHO PQT/VCP Implementation Guidance – Supporting data considerations for the use of novel bioassays

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15 Annex I. Survey templates

1. Baseline survey
2. ITNs baseline net listing and sampling
3. User acceptability
4. List A follow up surveys – Net attrition and fabric integrity
5. List B follow up surveys – Sampled ITNs for chemical content, physical characteristics, laboratory bioassays and semi-field studies

16 Annex II. Suggested table formats for summary results

16.1 Baseline quality checks

16.1.1 Table format for baseline quality check chemical analysis results

Table x. Baseline quality check chemical analysis results of ITNs received at [testing facility name] for [product name(s) and batch numbers [batch#1, batch#2, batch#3]]

[Product name 1]					
Sample ID (net and batch identification)	Number of net samples	Mean [AI name] content (g/kg)	RSD (%)	Mean [synergist name, or second AI] content (g/kg)	RSD (%)
[sample IDs Batch 1 Net1]		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-net variability]	[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-net variability]
[sample IDs Batch 1 Net2]					
[sample IDs Batch 1 Net3]					
[sample IDs Batch 1 Net4]					
[sample IDs Batch 1 Net5]					
Combined Batch [1] results		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]	[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]
[sample IDs Batch 2 Net1]					
[sample IDs Batch 2 Net2]					
[sample IDs Batch 2 Net3]					
[sample IDs Batch 2 Net4]					
[sample IDs Batch 2 Net5]					
Combined Batch [2] results		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]	[mean] ([SD][range lower limit] -[range upper limit])	[value showing the effect of intra-batch variability]
[sample IDs Batch 3 Net1]					
[sample IDs Batch 3 Net2]					

Table x. Baseline quality check chemical analysis results of ITNs received at [testing facility name] for [product name(s) and batch numbers [batch#1, batch#2, batch#3]]					
[sample IDs Batch 3 Net3]					
[sample IDs Batch 3 Net4]					
[sample IDs Batch 3 Net5]					
Combined Batch [3] results		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]	[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]
Combined results for all batches		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the inter-batch variability]	[mean] ([SD][range lower limit] -[range upper limit])	this value shows the inter-batch variability]
Add additional rows for additional products, as required					

16.1.2 Table format for baseline quality check bioassay results

Table x. Baseline quality check bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes												
Sample ID (net and batch identification)	Product/Fabric [A]				Product/Fabric [B]				Product/Fabric [C]			
	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)
[sample IDs Batch 1 Net1]												
[sample IDs Batch 1 Net2]												
[sample IDs Batch 1 Net3]												
[sample IDs Batch 1 Net4]												
[sample IDs Batch 1 Net5]												
Batch [1] combined results												

Table x. Baseline quality check bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes												
Sample ID (net and batch identification)	Product/Fabric [A]				Product/Fabric [B]				Product/Fabric [C]			
	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)
[sample IDs Batch 2 Net1]												
[sample IDs Batch 2 Net2]												
[sample IDs Batch 2 Net3]												
[sample IDs Batch 2 Net4]												
[sample IDs Batch 2 Net5]												
Batch [2] combined results												
[sample IDs Batch 3 Net1]												
[sample IDs Batch 3 Net2]												
[sample IDs Batch 3 Net3]												
[sample IDs Batch 3 Net4]												
[sample IDs Batch 3 Net5]												
Batch [3] combined results												
Combined results for all batches												
Add additional rows for additional products/fabrics if required												
NB. Present results for the negative control, positive control(s) and ITN under investigation. Additional rows/columns may be added for additional products/endpoints/species/strains.												

16.1.3 Table format for visual inspection results

Table x. Baseline quality check visual inspection results for test and reference items.							
Lot number	Bale number	Sampling position	Visual inspection				
			Fabric integrity, including seams	Colour	Symmetry	Material	Overall damage present?
Test product							
Reference product							
Add additional rows for additional products/lot numbers/bales/sampling positions as required							

* Adapted from (12)

16.1.4 Table format for study characteristics

Table x. Number of HH enrolled, number of study ITNs distributed and number of ITNs followed at each study timepoint for test and reference items.		
Characteristic	[Product 1]	[Product 2]
Number HH enrolled		
Number study nets distributed		
Number of nets followed per timepoint		
6 months		
12 months		
24 months		
36 months		
Number of nets not followed per timepoint		
6 months		
12 months		
24 months		
36 months		
Number of nets assessed for hole index/phi per timepoint		
6 months		
12 months		
24 months		
36 months		
Number of nets not assessed for hole index/phi per timepoint (due to attrition)		
6 months		
12 months		
24 months		
36 months		

* Adapted from (12)

16.1.5 Table format for HH consent results

Table x. HH consent results		
Consent indicators	[Product 1]	[Product 2]
Baseline		
Number of HH enrolled		
6 months		
Number of HH visited		
Number HH consented		
Consent results:		
• Consent given		
• Dwelling not found		
• Dwelling vacant		
• HH not visited		
• HH visited but not selected		
• Refused		
12 months		
Number of HH visited		
Number HH consented		
Consent results:		
• Consent given		
• Dwelling not found		
• Dwelling vacant		
• HH not visited		
• HH visited but not selected		
• Refused		
24 months		
Number of HH visited		
Number HH consented		
Consent results:		
• Consent given		
• Dwelling not found		
• Dwelling vacant		
• HH not visited		
• HH visited but not selected		
• Refused		
36 months		
Number of HH visited		
Number HH consented		
Consent results:		
• Consent given		
• Dwelling not found		
• Dwelling vacant		
• HH not visited		
• HH visited but not selected		
• Refused		

16.1.6 Table format for supplementary bioassay results

Table x. Supplementary bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes in [location]												
	[Species/strain 1]						[Species/strain 2]					
	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential Mean M24	OR (95% CI)	p	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential mean M24	OR (95% CI)	p
Unwashed ITN under investigation before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
20x washed ITN under investigation before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
12-months old ITN under investigation before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
24-months old ITN under investigation before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												

Table x. Supplementary bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes in [location]

	[Species/strain 1]						[Species/strain 2]					
	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential Mean M24	OR (95% CI)	p	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential mean M24	OR (95% CI)	p
36-months old ITN under investigation before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
Unwashed positive control before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
20x washed positive control before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
36-months old positive control before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
Unwashed ITN under investigation after [semi-field method]												
[Product/fabric A]												

Table x. Supplementary bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes in [location]

	[Species/strain 1]						[Species/strain 2]					
	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential Mean M24	OR (95% CI)	p	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential mean M24	OR (95% CI)	p
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
20x washed ITN under investigation after [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
12-months old ITN under investigation after [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
24-months old ITN under investigation after [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
36-months old ITN under investigation after [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												

Table x. Supplementary bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes in [location]

	[Species/strain 1]						[Species/strain 2]					
	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential Mean M24	OR (95% CI)	p	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential mean M24	OR (95% CI)	p
[Product/fabric D]												
Unwashed positive control after [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
20x washed positive control after [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
36-months old positive control after [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												

16.1.7 Table format for semi-field summary statistics

Table x. Summary statistics by arm and [endpoint(s)] for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]					
	Negative control	[Product A]	[Product B]	[Product C]	[Product D]
Unwashed					
N females entering					
N females per hut per night					
N females dead at 24 hours					
Mean M24 (95% CI)]					
N females [indicator] at [endpoint 2]					
Mean [endpoint 2] (95% CI)					
N blood fed females					
Blood feeding rate (%)					
Blood-feeding inhibition (%)					
20x washed					
N females entering					
N females per hut per night					
N females dead at 24 hours					
Mean M24 (95% CI)]					
N females [indicator] at [endpoint 2]					
Mean [endpoint 2] (95% CI)					
N blood fed females					
Blood feeding rate (%)					
Blood-feeding inhibition (%)					
12-months old					
N females entering					
N females per hut per night					
N females dead at 24 hours					

Table x. Summary statistics by arm and [endpoint(s)] for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]					
	Negative control	[Product A]	[Product B]	[Product C]	[Product D]
Mean M24 (95% CI)]					
N females [indicator] at [endpoint 2]					
Mean [endpoint 2] (95% CI)					
N blood fed females					
Blood feeding rate (%)					
Blood-feeding inhibition (%)					
24-months old					
N females entering					
N females per hut per night					
N females dead at 24 hours					
Mean M24 (95% CI)]					
N females [indicator] at [endpoint 2]					
Mean [endpoint 2] (95% CI)					
N blood fed females					
Blood feeding rate (%)					
Blood-feeding inhibition (%)					
36-months old					
N females entering					
N females per hut per night					
N females dead at 24 hours					
Mean M24 (95% CI)]					
N females [indicator] at [endpoint 2]					
Mean [endpoint 2] (95% CI)					
N blood fed females					
Blood feeding rate (%)					

Table x. Summary statistics by arm and [endpoint(s)] for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]					
	Negative control	[Product A]	[Product B]	[Product C]	[Product D]
Blood-feeding inhibition (%)					

16.1.8 Table format for secondary outcomes in semi-field studies

Table x. Summary statistics by arm and [endpoint(s)] for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]					
	Negative control	[Product A]	[Product B]	[Product C]	[Product D]
Unwashed					
N females entering					
N females per hut per night					
Deterrence (%)					
Total exiting					
% exiting (95% CI)					
Killing effect (%)					
Personal protection (%)					
20x washed					
N females entering					
N females per hut per night					
Deterrence (%)					
Total exiting					
% exiting (95% CI)					
Killing effect (%)					
Personal protection (%)					
12-months old					

Table x. Summary statistics by arm and [endpoint(s)] for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]					
	Negative control	[Product A]	[Product B]	[Product C]	[Product D]
N females entering					
N females per hut per night					
Deterrence (%)					
Total exiting					
% exiting (95% CI)					
Killing effect (%)					
Personal protection (%)					
24-months old					
N females entering					
N females per hut per night					
Deterrence (%)					
Total exiting					
% exiting (95% CI)					
Killing effect (%)					
Personal protection (%)					
36-months old					
N females entering					
N females per hut per night					
Deterrence (%)					
Total exiting					
% exiting (95% CI)					
Killing effect (%)					
Personal protection (%)					

16.1.9 Table format for non-inferiority analysis results for semi-field studies

Table x. Non-inferiority analysis for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]

	20x washed				36-month			
Indicator and reference	Target outcome	Δ for 7% difference	OR (95% CI)	Interpretation	Target outcome	Δ for 7% difference	OR (95% CI)	Interpretation
M24	Non-inferiority				Non-inferiority			
[endpoint 2] [active comparator]	Non-inferiority				Non-inferiority			
Blood feeding rate [active comparator]	Non-inferiority				Non-inferiority			